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SUBSTITUTE SHEET

## CLAIMS:

1. A method for changing the temperature of a sample from an initial temperature via an intermediate temperature to a final temperature, one of the initial and final temperatures being above the freezing point of said sample and the other being below the freezing point, the minimal dimension of the sample in each of two mutually perpendicular cross-sections exceeding 0.5 centimeters, and at least one of the cross-sections having an outer zone and an inner zone, the method comprising:
  - (i) changing the temperature of the sample by subjecting it to a temperature gradient from the initial temperature to the intermediate temperature until the temperature of the sample in at least one part of the outer zone equals the intermediate temperature whilst the temperature of the sample in the inner zone or in another part of the outer zone, spaced from said one part, is different from said intermediate temperature;
  - (ii) further changing the temperature of said sample by subjecting it to the intermediate temperature until the temperature of said sample in at least one cross-section is uniform and equals the intermediate temperature; and
  - (iii) changing the temperature of said sample until the majority of said sample is at the final temperature.
2. The method of Claim 1, wherein said sample is subjected in step (ii) to said intermediate temperature until the temperature of the sample equals said intermediate temperature.
3. The method according to anyone of Claims 1 or 25, wherein the changing of the temperature in step (i) is achieved by moving the sample through a region with ~~a~~ the temperature gradient from the initial temperature to the intermediate temperature, and the changing of the temperature in step (iii) is achieved by moving the sample through a region with a temperature gradient from the intermediate temperature to the final temperature.
4. The method of Claim 3, wherein said changing of the ambient temperature is at least partially gradual and is achieved at least partially by the gradual movement of said sample in the direction of a temperature gradient.

5. The method of Claim 4, wherein the changing of temperature in step (ii) is performed by placing said sample in a region with the intermediate temperature, said region having a length along the direction of the movement of said sample and said length is not less the length of the sample along said direction of movement.
- 5 6. The method according to Claim 5, wherein the changing of the temperature in step (i) is achieved by moving the sample through a region with a temperature gradient from the initial temperature to the intermediate temperature, and the changing of the temperature in step (iii) is achieved by moving the sample through a region with a temperature gradient from the intermediate temperature to the final  
10 temperature.
7. The method according to any one of Claims 5 and 6, wherein the sample has a leading end along the direction of movement and step (i) comprises:
- (1) moving the leading end of the into a region with a temperature gradient from the initial temperature to the intermediate temperature;
  - 15 (2) pausing the movement until seeding takes place at the leading end; and
  - (3) moving the sample through said region.
8. The method according to Claim 7, wherein the seeding in step (2) is achieved by introduction of liquid nitrogen to said leading end of the sample.
9. The method according to any one of Claims 5 to 8, wherein step (ii)  
20 comprises:
- (a) Moving the sample into the region with the intermediate temperature, until substantially the whole sample is within said region;
  - (b) Pausing the movement of the sample within said region until the temperature of the sample is substantially uniform throughout the sample and  
25 equals the intermediate temperature; and
  - (c) Moving the sample out of said region.
10. The method according to any of Claims 5 to 9, wherein the velocity of movement in step (i) is equal to the velocity of movement in step (iii).
11. The method according to any of Claims 5, to 9, wherein the velocity of  
30 movement in step (i) is different from the velocity of movement in step (iii).

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12. The method according to any of Claims 5 to 11, wherein the volume of the sample exceeds 5 milliliters.
13. The method according to Claim 12, wherein the volume of the sample is 12 milliliters or more.
- 5 14. The method according to Claim 12, wherein the volume of the sample is 50 milliliters or more.
15. The method according to any one of Claims 5, 6, 9, 10, 11 and 12, wherein the sample comprises blood cells.
16. The method according to any one of Claims 5, 6, 9, 10, 11, and 12, wherein  
10 the sample comprises plasma.
17. The method according to any one of Claims 5, 6, 9, 10, 11, and 12, wherein the sample comprises one or more embryos.
18. The method according to any one of Claims 5, 6, 9, 10, 11 and 12 wherein the sample comprises semen.
- 15 19. The method according to Claim 18, wherein the sample comprises more than one insemination quota.
20. The method according to any one of Claims 18 and 19, wherein the sample is substantially a whole ejaculate of semen.
21. The method according to any one of Claims 18, 19 and 20, wherein the  
20 initial temperature is above the freezing point of the sample and the final temperature is below said freezing point, said method further comprising after step (iii):
- (iv) thawing the sample;
- (v) dividing said sample to aliquots; and
- 25 (vi) freezing at least one of said aliquots.
22. The method according to Claim 21, wherein step (v) comprises diluting of the thawed at least one aliquot with an extender before dividing it to smaller aliquots.
23. The method according to any one of Claims 21 or 22, wherein at least one of  
30 the aliquots comprises one or more insemination quotas.

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24. The method according to any one of Claims 21 or 22, wherein the in step (v) the sperm is divided into aliquots in accordance with a trait of the semen.
25. The method according to Claim 24, wherein said trait is the sex chromosome of the sperm.
- 5 26. The method according to any one of Claims 21, to 25, wherein the semen comprises sperm and extra-cellular fluid, and wherein step (iv) is terminated when the sample reaches a temperature wherein the extra-cellular fluid is thawed while the sperm is chilled.
27. The method according to any one of Claims 18, 19 and 20, wherein the  
10 sample comprises sperm, seminal plasma and an added extender.
28. The method according to Claim 27, wherein the ratio in the sample of the sperm number to the volume of the seminal plasma before addition of the extender is the same as the ratio of the sperm number to the volume of the seminal plasma in the raw ejaculate.
- 15 29. The method according to any one of Claims 18, 19 and 20, wherein the sample comprises semen collected from more than one donor.
30. The method according to any one of Claims 18, 19, 20 and 29, wherein the sample comprises two or more insemination quotas.
31. The method according any one of Claims 1 to 30, wherein the sample is  
20 mammalian.
32. The method according to Claim 31, wherein the sample is taken from humans.
33. The method according to Claim 31, wherein the sample is taken from non-humans.
- 25 34. The method according to Claim 33, wherein the sample is taken from bovines.
35. The method according to Claim 33, wherein the sample is taken from equines.
36. The method according to Claim 35, wherein the sample is taken from  
30 horses.

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37. The method according to Claim 35, wherein the sample is taken from donkeys.
38. The method according to Claim 33, wherein the sample is taken from ovines.
- 5 39. The method according to Claim 38, wherein the sample is taken from sheep.
40. The method according to Claim 38, wherein the sample is taken from goats.
41. The method according to Claim 33, wherein the sample is taken from porcines.
42. The method according to any one of Claims 1 to 30, wherein the sample is  
10 taken from avians.
43. The method according to Claim 42, wherein the sample is taken from turkeys.
44. The method according to Claim 42, wherein the sample is taken from fowl.
45. The method according to Claim any one of Claims 1 to 30, wherein the  
15 sample is taken from fish.
46. A device for changing the temperature of a sample, said device comprising:
- a track;
  - a mechanism for moving the sample along said track.
  - temperature control means for imposing a temperature gradient  
20 along a first area along said track;
  - temperature control means for imposing a constant temperature along a second area along said track, such that the length of said second area along the track would be at least equal to the length of the sample along said track; and
  - 25 - temperature control means for imposing a temperature gradient along a third area along said track;
47. The device according to Claim 46, wherein the mechanism for moving the sample along said track is capable of moving the sample at a constant velocity, along the track in said first and third areas.

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48. The device according to any one of Claims 46 and 47, wherein the mechanism for moving the sample along said track is capable of moving the sample along the track in said first and third areas at different velocities one from the other.

49. The device according to Claim 48, wherein the mechanism for moving the sample along said track is capable of stopping the movement when the sample is within the second area and later resuming the movement.

50. The device according to any one of Claims 46, 47, 48 and 49, wherein the minimal dimension of the sample in each of two mutually perpendicular cross-sections exceeds 0.5 centimeters.

51. The device according to any one of Claims 46, 47, 48, 49, and 50, wherein the sample has a volume of at least 5 milliliters.

52. The device according to Claim 51, wherein the sample has a volume exceeding 12 milliliters.

53. The device according to Claim 51, wherein the sample has a volume exceeding 50 milliliters.

54. The device according to any one of Claims 1 to 53, wherein the initial temperature is above the freezing point of the sample and the final temperature is below said freezing point.

55. A device for changing the temperature of a sample, the minimal dimension of the sample in each of two mutually perpendicular cross-sections exceeding 0.5 centimeters, said device comprising:

- a track;
- a mechanism for moving the sample in a direction along said track;
- temperature control means for imposing a temperature gradient along a first area along said track;
- temperature control means for imposing a constant temperature along a second area along said track, such that the length of said second area along the track would suffice to allow the sample, at each cross-section taken perpendicularly to said direction to reach the

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intermediate temperature by the time it is moved out of said second area;  
and

- temperature control means for imposing a temperature gradient along a third portion of said track.

5 56. The device according to any one of Claim 55, wherein the sample has a volume of at least 5 milliliters.

57. The device according to Claim 56, wherein the sample has a volume exceeding 12 milliliters.

58. The device according to Claim 56, wherein the sample has a volume  
10 exceeding 50 milliliters.

59. A device for changing the temperature of a sample, the minimal dimension of the sample in each of two mutually perpendicular cross-sections exceeding 0.5 centimeters, said device comprising:

- a track;
- 15 – a mechanism for moving the sample along said track;
- temperature control means for imposing a temperature gradient along a first area along said track; and
- temperature control means for imposing a constant temperature along a second area along said track, such that the length of said second  
20 area along the track would be at least equal to the length of the sample along said track

60. The device according to Claim 59, wherein the sample has a volume of at least 5 milliliters.

61. The device according to Claim 60, wherein the sample has a volume  
25 exceeding 12 milliliters.

62. The device according to Claim 60, wherein the sample has a volume exceeding 50 milliliters.

63. A method for preservation of semen comprising collecting a whole ejaculate of semen from a single donor and freezing said whole ejaculate as a single sample.

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64. The method according to Claim 63, wherein the whole ejaculate is the sperm rich fraction.

65. A method for double-freezing preservation of semen comprising:

(A) freezing the semen in one or more aliquots;

5 (B) thawing at least one aliquot;

(C) dividing said thawed aliquot to smaller aliquots; and

(D) freezing at least one of said smaller aliquots.

66. The method according to Claim 65, wherein step (C) comprises diluting of the thawed aliquot with an extender before dividing it to smaller aliquots.

10 67. The method according to any one of Claims 65 and 66 wherein the one or more aliquots of step (A) each comprise more than one insemination quota.

68. The method according to any one of Claims 65 and 66, wherein the one or more aliquots of step (A) each comprise a whole ejaculate of a single male.

15 69. The method according to any one of Claims 65 to 68, wherein the smaller aliquots are each equal to an insemination quota.

70. The method according to any one of Claims 65 to 69, wherein the semen comprises sperm and extra-cellular fluid, and wherein step (B) is terminated when the sample reaches a temperature wherein the extra-cellular fluid is thawed while the sperm is chilled.

20 71. The method according to Claim 70, wherein the termination of step (B) comprises transferring the sample to a solution with said temperature.

72. The method according to any one of Claims 70 and 71, wherein said temperature is 5°C.

25 73. The method according to any one of Claims 65 to 72, wherein in step (C) the sperm is divided into aliquots in accordance with a trait of the semen.

74. The method according to Claim 73, wherein said trait is the sex chromosome of the sperm.

75. A method for preservation of semen comprising:

(I) adding an extender to a raw semen sample; and

30 (II) freezing said sample.



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76. A method for preservation of semen comprising collecting semen from more than one donor and freezing it as a mixture.

77. The method according to Claim 76, wherein the semen comprises two or more insemination quotas.